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Cultivation shapes genetic novelty in a globally important invader

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Abstract

***Acacia saligna* is a species complex that has become invasive in a number of countries worldwide where it has caused substantial environmental and economic impacts. Understanding genetic and other factors contributing to its success may allow managers to limit future invasions of closely related species. We used three molecular markers to compare the introduced range (South Africa) to the native range (Western Australia). Nuclear markers showed that invasive populations are divergent from native populations and most closely related to a cultivated population in Western Australia. We also found incongruence between nuclear and chloroplast data that, together with the long history of cultivation of the species, suggest that introgressive hybridization (coupled with chloroplast capture) may have occurred within *A. saligna*. While we could not definitively prove introgression, the genetic distance between cultivated and native *A. saligna* populations was comparable to known interspecific divergences among other *Acacia* species. Therefore, cultivation, multiple large-scale introductions and possibly introgressive hybridization have rapidly given rise to the divergent genetic entity present in South Africa. This may explain the known global variation in invasiveness and inaccuracy of native bioclimatic models in predicting potential distributions.**

Keywords: *Acacia saligna*, biological invasions, chloroplast capture, genetic novelty, introgression, tree invasions

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Introduction

Biological invasions tend to promote rapid evolution because of the introduction process and the novel selection pressures that arise in the introduced range (Prentis *et al.* 2008). Indeed, postintroduction establishment has been associated with a number of genetic characteristics, including high genetic diversity (e.g. reed canarygrass, Lavergne & Molofsky 2007; European paper wasp, Johnson & Starks 2004; European starling, Rollins *et al.* 2009), increased phenotypic plasticity (e.g. Chinese tallow tree, Zou *et al.* 2009; anolis lizards, Kolbe *et al.* 2007) and novel genotypes arising from hybridization (e.g. *Tamarix* spp., Gaskin & Kazmer 2009; freshwater sculpin, Nolte *et al.*, 2005).

Cultivation plays an important role in determining the influence of such processes for two main reasons. First, species used in horticulture and silviculture are typically introduced on multiple occasions, in large quantities, and are planted widely with resources to facilitate establishment, that is, there is likely to be high propagule pressure, high genetic diversity and opportunities for novel genetic combinations to arise (Ellstrand & Schierenbeck 2000; Wilson *et al.* 2009). Second, breeding and selection can favour traits associated with invasiveness, for example, fast growth rates and robustness to adverse environmental conditions (e.g. Richardson 1998; Paynter *et al.* 2003; Richardson & Rejmánek 2011).

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A species' native phylogeographic structure or evolutionary history can similarly influence the introduced genetic signature as it defines the genetic pool from which the invader is drawn (Taylor & Keller 2007; Le Roux *et al.* 2011), and so to some extent it affects the degree to which cultivation and introduction dynamics can create new genetic entities. This is of particular importance for species complexes, as their introduction dynamics and native phylogeographic structure can act in concert to determine their introduced intraspecific diversity, the opportunity for intraspecific hybridization (admixture) or the development of novel genotypes. Indeed, sympatric introductions of different genotypes have produced entities that are more phenotypically plastic, with greater fitness than their native counterparts (e.g. Durka *et al.* 2005; Sun *et al.* 2007; Thompson *et al.* 2011).

Woody plants used in silviculture and agriculture have been widely distributed and cultivated for centuries, in many instances resulting in invasive populations (Richardson 1998; Thuiller *et al.* 2006; Richardson & Rejmánek 2011). Australian acacias (1012 recognized species native to Australia, previously grouped in *Acacia* subgenus *Phyllodineae*) are a model group for the study of woody plant invasions (Richardson *et al.* 2011). More than a third of taxa in the group (386 species) have been introduced outside their natural range, and many of them have been repeatedly introduced to the same region, or to multiple regions (Richardson *et al.* 2011). A number of species display very high levels of intraspecific genetic diversity and structure in their native ranges (Le Roux *et al.* 2011), allowing repeated tests of the influence of the invasion dynamics of a species on the genetic signature in the introduced range. Their introduction histories are relatively well documented (Griffin *et al.* 2011; Le Roux *et al.* 2011; Richardson *et al.* 2011), providing definitive records on introduction mode and date. Furthermore, many species were selected for introduction because of fast growth rates, their ability to survive in adverse conditions and incidentally their weediness (Griffin *et al.* 2011).

Acacia saligna (Labill.) H. L. Wendl., a species complex native to Western Australia, is one of the most frequently exported Australian acacia taxa (Griffin *et al.* 2011) and now occurs in at least 20 countries worldwide (Richardson & Rejmánek 2011; Richardson *et al.* 2011). It has been used for timber, as an ornamental plant, as a source of fodder, fuel, fibre and tannin, and for erosion control (Orwa *et al.* 2009; Kull *et al.* 2011). *Acacia saligna* is an allogamous, diploid ($2n = 26$, Ghimpu 1929), insect-pollinated shrub or tree (Atchison 1948; Millar *et al.* 2008; Gibson *et al.* 2011) that bears hermaphroditic, globular inflorescences (Maslin & McDonald 2004). The species displays high levels of ecological, phenotypic and genetic variation throughout its native range (Maslin 1974; Maslin & McDonald 2004). This variation is not easily ascribed to subspecific entities, and the number and categorization of taxonomically distinct entities has been a matter of debate (Maslin 1974; Maslin & McDonald 2004; George *et al.* 2006; Millar *et al.* 2008, 2011). As no classification has been formalized, throughout this manuscript we refer to both the most recent morphological treatment: (i) subspecies *'lindleyi'* ('typical' variant); (ii) subspecies *'stolonifera'* ('forest' variant); and (iii) subspecies *'saligna'* ('cyanophylla' variant) and 4) subspecies *'pruinescens'* ('Tweed River' variant, Maslin & McDonald 2004; worldwidewattle.com), and the three main genetic groups identified by Millar *et al.* (2011). The only difference between the two treatments is that the molecular treatment does not differentiate between subspecies *'saligna'* and *'pruinescens'*. Each informal subspecies has differing ecological traits (seed set, reproductive success and biomass production) and a preference for particular environmental conditions, for example, the 'cyanophylla' variant prefers deep sandy soils, while the 'typical' variant is common on seasonally dry water courses and around granite rocks (Maslin & McDonald 2004). Such ecological characteristics might be expected to persist in the introduced range and aid or impede invasive success.

Acacia saligna was introduced to South Africa in about 1833 for dune stabilization and ornamental purposes (Roux 1961; Shaughnessy 1980) and was later used as a wood and tannin source. From 1833 to 1890, over 50 million seeds were distributed and several thousand seedlings planted (Roux 1961). These populations have since expanded considerably, and *A. saligna* now extends over some 1.8 million ha of natural and semi-natural land in South Africa (Le Maitre *et al.* 2000). Despite relatively detailed records of the time, number and locations of introductions of *A. saligna* to South Africa, the source of seeds and the subspecific identity of invasive populations remain unknown. Various control measures have been used including mechanical, chemical and biological control (Wood & Morris 2007). While the introduction of classical biological control agents has substantially reduced the density of infestations (Impson *et al.* 2011), *A. saligna* remains one of the most costly invasive plants in South Africa (van Wilgen *et al.* 2012).

Our overall goal in this study was to improve our understanding of *A. saligna* invasions by examining which subspecific entities are present in South Africa using pure native lineages (referred to as reference populations from here on) from Western Australia (Millar *et al.* 2011). Ultimately, we hope this will guide future biological control programs and provide insight into the

invasion dynamics of other invasive acacia species already present in South Africa and other regions. Specifically, we use DNA sequence and microsatellite variation to: (i) place the invasive populations within a framework of spatial genetic structure among different subspecies/genetic lineages of *A. saligna* in their native range; (ii) compare levels of genetic diversity in invasive populations of *A. saligna* to those in the native range; (iii) relate the population genetic structure of invasive *A. saligna* to its known invasion dynamics; and (iv) discuss the implications of our findings for the management of *A. saligna* in South Africa.

Materials and methods

Sampling design and DNA isolation

Phyllode material of *A. saligna* was collected from 163 individuals from the introduced range in South Africa. We also included a single native individual from Wilbinga, as well as individuals introduced to New South Wales and South Australia (five individuals), Israel (two individuals) and Spain (one individual, Table 1). Because of low sample sizes, these (non-South African) accessions were only included in our phylogeo-

Table 1 Microsatellite genetic diversity indices for native and introduced populations of *Acacia saligna*

Locality	ID	*N*	N_A	N_{PA}[§]	Ho	He	Latitude	Longitude
Native								
Western Australia								
Parkeyerring[†]	PAR	5	3.6	4	0.439	0.683	−33.362	117.356
Ravensthorpe[†]	RAV	5	3	2	0.439	0.567	−33.258	119.751
Wellesley	WEL	21	3.1	4	0.443	0.475	−33.148	115.742
Busselton	BUS	21	4.6	1	0.451	0.522	−33.661	115.358
Tuart Forest	TUA	11	3.7	2	0.394	0.498	−33.54	115.508
Dinninup[†]	DIN	28	5.2	7	0.415	0.603	−33.813	116.534
Wilbinga[†]	WIL	1	—	—	—	—	−31.438	115.663
Wanneroo[‡]	WAN	13	3.1	1	0.338	0.428	−31.438	115.663
Leshnault Inlet[‡]	WEI	14	3.3	2	0.38	0.391	−33.218	115.693
Mount Ney[‡]	MTN	14	3.5	7	0.462	0.52	−33.398	122.466
Preston[‡]	PRE	14	3.6	3	0.365	0.529	−33.529	115.97
Muntagin[‡]	MUN	15	3.7	6	0.482	0.484	−31.758	118.583
Tweed River[‡]	TWR	14	2.9	2	0.36	0.386	−34.58	116.492
Wickepin[‡]	WIC	15	4.4	5	0.468	0.544	−32.63	117.384
Boyatup Hill[‡]	BOY	12	3.2	4	0.408	0.444	−33.738	123.044
Introduced								
South Africa								
Cinsta[†]	CIN	31	3.8	3	0.29	0.392	−32.845	28.113
Ebenhaezer[†]	EBE	24	3.3	5	0.334	0.434	−31.586	18.242
Breede River[†]	BRE	14	3.5	3	0.412	0.485	−34.12	20.034
Port Alfred	PA	28	2.8	1	0.305	0.363	−33.554	26.893
Sedgefield	SED	15	3.3	2	0.393	0.496	−34.011	22.779
Jeffrey's Bay	JBAY	33	4	5	0.288	0.499	−34.052	24.922
Albertinia*	ALB	18	3.2	1	0.299	0.375	−34.137	21.699
Australia								
Tintinara, South Australia*	TIN	2	—	—	—	—	−35.921	140.101
Sydney, New South Wales*	SYD	3	—	—	—	—	−33.765	151.233
Eurasia								
Israel	ISR	2	—	—	—	—	31.736	34.617
Spain*	SPA	1	—	—	—	—	36.72	−4.42

*Populations without ETS data.

[†]Populations without trnQ-5'rps16 data.

[‡]reference populations of *A. saligna* from Millar *et al.* (2011, 2012).

[§]Calculated in GENALEX.

N, number of individuals genotyped/sequenced at site; N_A, mean number of alleles; N_{PA}, number of private alleles; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity.

graphic data sets. For comparisons between South Africa and the native range, we used DNA from eight reference populations included in Millar *et al.* (2011) to identify intraspecific variants (Table 1); these are considered to represent pure native lineages of *A. saligna*. Collections were also made from seven additional populations from Western Australia that did not have definitive subspecies identifications. In addition, a collection was made at the original locality (Busselton) from which a fungal biological control agent, *Uromycladium tepperianum,* was collected for release in South Africa (Morris 1991). We also downloaded one ETS sequence from GenBank for a cultivated specimen of *A. saligna* that originated from a glasshouse specimen in Canberra, Australian Capital Territory, Australia (GenBank number: FJ868448.1; herbarium specimen number: CANB 634053.1). Phyllode material was dried and stored on silica gel until DNA extraction. Genomic DNA was extracted using a modified cetyltrimethyl ammonium bromide method (Doyle & Doyle 1990) with the addition of 0.2 M sodium sulphite to the extraction and wash buffers following Byrne *et al.* (2001).

DNA sequencing and data analysis

One nuclear (external transcribed spacer, Brown *et al.* 2008) and one chloroplast (trnQ—5′rps16, Shaw *et al.* 2007) gene were amplified for all accessions where possible. See Appendix S1 in the Supporting information for amplification conditions.

Sequence data were aligned and edited using BioEdit v. 7.0.5.3 (Hall 1999). DnaSP v.5 (Librado & Rozas 2009) was used to identify different ETS sequences and calculate the average number of haplotypes (N_H), haplotype diversity (h) and nucleotide diversity (p) for the native and introduced ranges. For nDNA, we used ModelTest v.3.7 to determine the best-fit nucleotide substitution model (Posada & Crandall 2001) under the Akaike Information Criterion (AIC). Maximum likelihood analysis was conducted in PAUP* v.4b10 (Swofford 1999), using the TPM1uf model selected by ModelTest (Kimura 1981), and the heuristic search option. Support for internal branches was evaluated using 10 000 bootstrap replicates (Felsenstein 1985). nDNA phylogenetic reconstructions were rooted using two closely related species (*Acacia cupularis* and *Acacia rostellifera*, GenBank numbers: JF420247 and JF420272, respectively) known to be sister to *A. saligna* (see Miller *et al.* 2011). Population pairwise Φ_{ST} was calculated with 10 000 permutations in Arlequin v.3.5 (Excoffier & Lischer 2010). To assess genetic differentiation among sampling sites, we conducted a hierarchical analysis of molecular variance (amova; Excoffier *et al.* 1992) in Arlequin using 10 000 permutations. Relationships among the trnQ—5′rps16 haplotypes were examined using statistical parsimony to reconstruct haplotype networks generated at the 95% connection limit with TCS v.1.21 (Clement *et al.* 2000). Because of the low resolution present within the trnQ—5′rps16 region for *A. saligna*, we did not conduct further analyses to assess population structure (i.e. population pairwise Φ_{ST}), or the distribution of genetic variation (i.e. amova).

Microsatellite genotyping and data analysis

Ten nuclear microsatellite loci previously developed and characterized for *A. saligna* (Millar & Byrne 2007) were PCR-amplified in two separate multiplex reactions (five loci per multiplex) for each sample. Populations that had <5 individuals were not genotyped (populations from Wilbinga, South Australia, New South Wales, Spain and Israel). Each 10 μL reaction contained 0.25 U Taq polymerase (KapaBiosystems, Cape Town, South Africa), 1.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.4), 50 mM KCl, 0.2 mM of each dNTP, 5 μM of each primer and ~10 ng/μL genomic DNA. Thermocycling consisted of initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 15 s, 56 °C for 30 s and 72 °C for 10 s; no final extension was required. PCR fragments were separated on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using GeneScan™-500 (−250) as an internal size standard (Applied Biosystems). Allele sizes were visualized and scored using GeneMarker® v1.95 (SoftGenetics LLC®, Pennsylvania, USA).

Isolation by distance. Recent studies have suggested that the presence of strong isolation by distance (IBD) in microsatellite data can lead to incorrect deductions on the history of populations (Guillot *et al.* 2009). Consequently, we chose to test for IBD prior to further tests of genetic diversity and population structure. IBD analyses were computed for South Africa and Western Australia separately using Mantel tests and the online resource IBDWS v 3.16 (Jensen *et al.* 2005). For these, matrices of pairwise genetic distances (F_{ST} values calculated in arlequin) were plotted against geographical distances (Euclidian distances calculated in genalex v 6.4). The upper and lower 95% confidence limits were calculated from 10 000 permutations.

Genetic diversity. For the combined native and introduced data set, microsatellite data were tested for departures from Hardy–Weinberg equilibrium using 1 000 000 steps in the Markov chain in arlequin (Excoffier & Lischer 2010). We also tested for linkage disequilibrium for all pairs of loci in arlequin. For a broad overview of within-population genetic diversity parameters, we com-

pared the native (Western Australia) and introduced range (South Africa) by calculating the total number of alleles (N_A), allelic richness (R_S), mean observed and expected heterozygosities (H_E and H_O), the fixation index (F_{ST}) and the inbreeding coefficient (F_{IS}) in FSTAT v. 2.9.3.2 (Goudet 2001). FSTAT was used as it compensates for unequal sample sizes between populations. For a finer-scale analysis of diversity within individual populations, mean values of the following parameters were computed for polymorphic loci in ARLEQUIN (Excoffier & Lischer 2010): number of alleles (N_A), observed and expected heterozygosity (H_O and H_E) and inbreeding coefficients (F_{IS}). We also calculated the mean number of private alleles per population (P_A) in GENALEX (Peakall & Smouse 2006).

Population genetic structure. Several Bayesian clustering algorithms are available to determine the most likely number of biological populations or genetic demes (K, see Guillot *et al.* 2009 for a review of methods and software), each with advantages and drawbacks (e.g. Rowe & Beebee 2007). We used three different, spatially explicit Bayesian clustering algorithms. We chose spatially explicit models that incorporate admixture in all cases as these models are more robust than models that do not incorporate admixture and are better able to identify the optimal number of genetic clusters (François & Durand 2010). The three models employed were implemented in STRUCTURE v 2.3.2 (Falush *et al.* 2007), GENELAND v 3.1.4 (Guillot *et al.* 2005) and TESS v. 2.3.1 (Chen *et al.* 2007). For more details on model parameters and settings, refer to Appendix S2 in the Supporting information. To assess the effect allelic associations might have on genetic clustering of populations and regions (e.g. see Rosenthal *et al.* 2008), we compared both the number of private alleles in native and introduced ranges and identified differences in allelic frequencies that exceeded 10% between ranges in GENALEX.

Comparative genetic distances between species and subspecies. To compare the divergence present within species and subspecies in the genus *Acacia* (subgenus *Phyllodineae*) to the divergence present within *A. saligna*, we downloaded available ETS DNA sequence data from GenBank. For species comparisons, we selected one of the closest relatives of *A. saligna*, *A. rostellifera* (Miller *et al.* 2011). For subspecies comparisons, we selected *A. longifolia* (subspecies *sophorae* and subspecies *longifolia*). A matrix of pairwise genetic distances was calculated using DNADist in BIOEDIT (Hall 1999).

Visualization of genetic distance. To provide further support for the genetic groups inferred by phylogenetic reconstructions, we plotted pairwise genetic distances for the nuclear and chloroplast DNA sequences using a non-metric multidimensional scaling analysis (NMDS). Pairwise genetic distances between all individuals for the ETS and trnQ-5'rps16 genes were calculated in BIOEDIT (Hall 1999). Accessions were clustered using NDMS and the 'ratio + bounds' setting in PERMAP v. 11.8a (Heady & Lucas 2007), and a highly accurate convergence value of 0.000005. Ten iterations were conducted, where each new iteration was initiated manually when the objective function moved towards a minimum value. Proximity coordinates for each individual were obtained from the solution with the lowest objective function value and plotted in R (R Development Core Team, 2004) using the 'car' package (Fox & Weisberg 2011).

Spatial distribution of genetic diversity. To determine the distribution of genetic variation (in the nuclear microsatellites and nDNA sequence data) between groups of individuals or populations at different scales, we conducted an analysis of molecular variance (AMOVA) using ARLEQUIN (Excoffier & Lischer 2010). We partitioned total genetic variance at three hierarchical levels—among invasive and native regions, among populations within regions, and within populations. The degree of population differentiation and spatial variation was also estimated by computing population pairwise F_{ST} values for all populations in Western Australia and South Africa independently. This analysis was conducted in ARLEQUIN where the F_{ST} significance levels were assessed using a Bonferroni adjustment for multiple comparisons (Weir 1996). Lastly, the distribution of genotypes in the native and invasive range was further assessed by combining genetic and geographic distance for all sampled individuals of *A. saligna* using a covariance standardized principal coordinate analysis (PC_OA) in GENALEX (Peakall & Smouse 2006) and 1000 permutations.

Results

Genetic diversity

There was no evidence of significant IBD in either the native ($r^2 = 0.0255$, $P = 0.9245$) or introduced ($r^2 = 0.0009$, $P = 0.5510$) ranges of *A. saligna* (Appendix S3—Fig. S1, Supporting information). Consequently, the effects of IBD were not considered in further analyses. Only two pairs of loci displayed significant levels of linkage disequilibrium ($P = 0.001$, data not presented).

All 10 microsatellite loci were found to be polymorphic. At these loci, there was a larger number of alleles (N_A), higher levels of allelic richness (R_S) and more unbiased gene diversity (H_S) in native populations compared to introduced populations, whereas introduced

populations were more inbred and had less differentiation than native populations (Table 2, Appendix S3—Table S1, Supporting information).

Native genetic structure

The clustering algorithms varied in the optimal number of native genetic clusters: $K_{native} = 3$ for STRUCTURE (Fig. 1, Appendix S3—Fig. S2, Supporting information), $K_{native} = 5$ for TESS (Appendix S3—Fig. S3, Supporting information) and $K_{native} = 6$ for GENELAND (Appendix S3—Fig. S4, Supporting information). Despite this incongruence, the most frequently retrieved genetic cluster (i.e. most dominant) in all analyses was consistent with the findings of Millar *et al.* (2011) and morphological identification of herbarium specimens by Bruce Maslin (*Acacia* expert, Department of Environment and Conservation, Western Australia). This cluster included individuals of *A. saligna* subspecies *'saligna'* and subspecies *'pruinescens'* (Figs. 1, Appendix S3—Figs S2, S5, Supporting information) and is consistent with the findings of Millar *et al.* (2011), that is, subspecies *'saligna'* and *'pruinescens'* are indistinguishable based on microsatellite data.

Table 2 Overall microsatellite genetic diversity indices for the native and introduced range of *Acacia saligna*

	R_S	H_S	H_O*	F_{IS}*	F_{ST} *
Native	1.490	0.506	0.414	0.181	0.330
Invasive	1.452	0.457	0.310	0.322	0.132

*$P < 0.05$.
R_S, allelic richness; H_S, unbiased gene diversity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient; and F_{ST}, among-population differentiation.

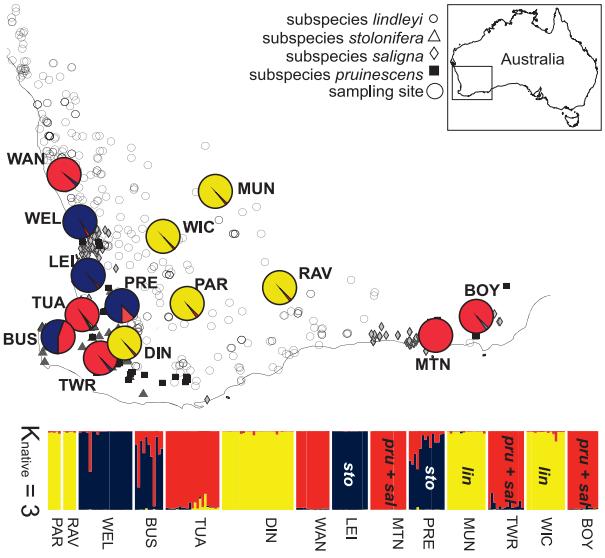

Fig. 1 Bayesian assignment of native genetic groups within the *Acacia saligna* complex, overlaid with known native distribution records for each of the four subspecies. Distribution records are based on morphological identification and were obtained from Australia's Virtual Herbarium online database (avh.rbg.vic.gov.au, accessed on 1 October 2010). Membership of each individual's genome (q_i) to the three indentified genetic clusters is indicated by vertical bars. Pie charts show overall genotype assignment for each population to particular genetic clusters. Reference populations of known informal subspecies were labelled according to Millar *et al.* (2011, 2012): lin (subspecies *'lindleyi'*), sto (subspecies *'stolonifera'*) and pru+sal (subspecies *'pruinescens'* and subspecies *'saligna'*).

Assignments of native populations by STRUCTURE (Fig. 1, Appendix S3—Fig. S2, Supporting information) and TESS (Appendix S3—Fig. S3, Supporting information) were congruent for the majority of sites. The clusters retrieved were similar to those identified by Millar *et al.* (2011). We used reference populations from Millar *et al.* (2011) to assign subspecies names to each cluster: cluster 1—*A. saligna* subspecies *'saligna'*; cluster 2—*A. saligna* subspecies *'lindleyi'* and cluster 3—*A. saligna* subspecies *'stolonifera'*. Overall, these groups were consistent with the relationships resolved by maximum likelihood based on ETS data (Fig. 2). GENELAND (Appendix S3—Fig. S4, Supporting information) gave somewhat different results—it identified a greater degree of genetic structure and did not identify any substantially mixed populations. It assigned the majority of populations (eight of 14) to a single cluster incongruent with STRUCTURE and TESS and identified a number of geographically localized populations (Muntagin, Leschnault Inlet, Wanneroo and Ravensthorpe) that were assigned to unique genetic clusters.

All 10 microsatellite loci yielded alleles with frequencies that differed by more than 10% between the native and introduced ranges (Appendix S3—Fig. S6, Supporting information). Of these 32 alleles, seven were found only in native populations (i.e. they were unique to Western Australia).

DNA sequence variation and phylogeography

The 485 bp ETS alignment contained a total of 109 polymorphic sites, 49 of which were parsimony-informative. Within the 50 individuals, we identified 27 distinct sequences, with 12 sequences unique to Western Australian, 12 sequences unique to South Africa and two sequences shared between regions. The remaining sequence was unique to South Australia (see FJ868448.1, Appendix S3—Fig. S7C, Supporting information). A number of native gene sequences were restricted to single populations in the native range (Appendix S3—Fig. S7A, Supporting information), which was not

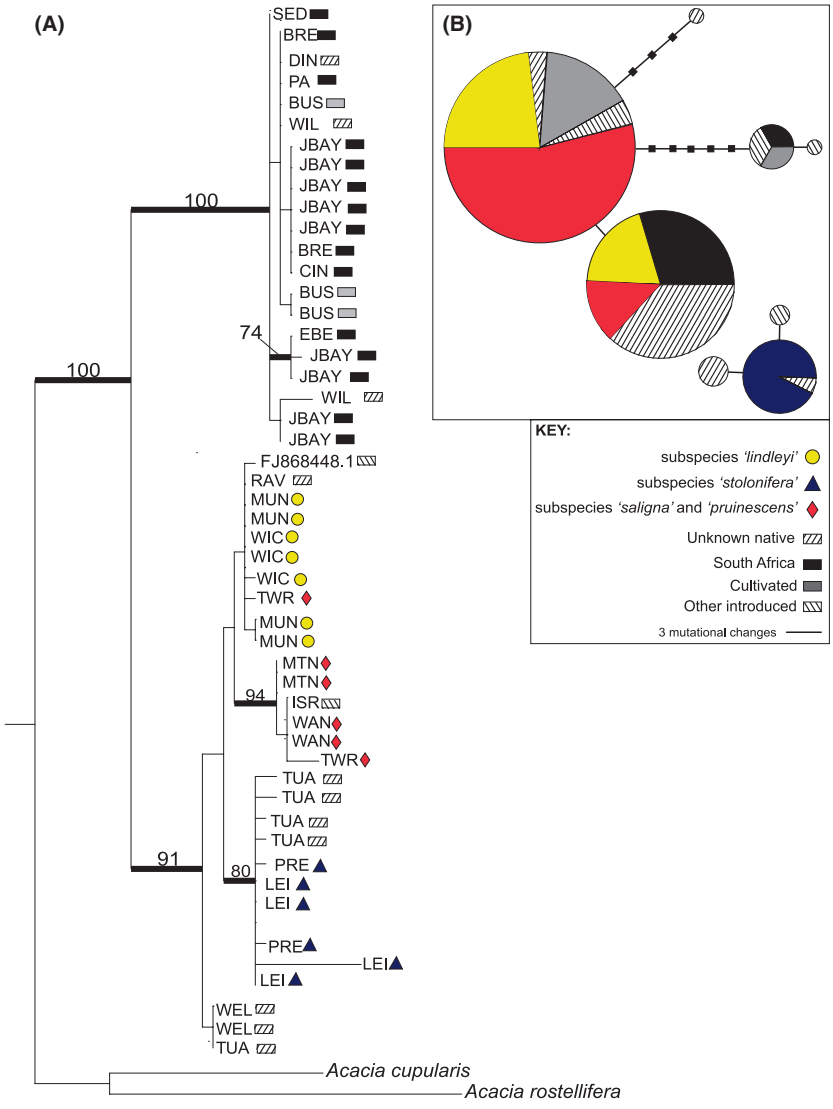

Fig. 2 Phylogenetic relationships within and among native and introduced populations of *Acacia saligna* based on (A) nDNA [maximum likelihood (CI = 0.950, RI = 0.981)] and (B) cpDNA (parsimony haplotype network, inset). In (A), all individuals are labelled by population name, and symbols indicate the subspecies identified in Millar *et al.* (2011, 2012). Tree branch lengths are scaled according to genetic distance, and bold branches represent strongly supported relationships (nodal support > 70). In (B), the shading differentiates between native and introduced populations. For both analyses, native subspecies were identified in Millar *et al.* (2011, 2012), while introduced populations are labelled by country or state of origin.

the case for introduced populations (Appendix S3—Fig. S7B, Supporting information). The most common DNA sequence in Western Australia was restricted to two geographically adjacent populations (Muntagin and Wickepin) in the north-eastern part of the native range of *A. saligna* (Appendix S3—Fig. S7A, Supporting information). These populations were identified as *A. saligna* subspecies *'lindleyi'* during Bayesian clustering (Fig. 1).

Sequence variation identified a number of features that were congruent with the nuclear microsatellite clustering of native populations. Individuals collected in populations that displayed mixed affinities in nuclear microsatellite clustering also lacked phylogenetic affinity in well-supported clades (notably the Busselton population, Fig. 2). The ETS phylogeny identified two main clades in the native range of the *A. saligna* species complex, while evidence for a third cluster containing all individuals representative of *A. saligna* subspecies *'lindleyi'* was present, but did not have significant support (nodal support < 70, Fig. 2). These results were not in complete agreement with the microsatellite structure identified by the STRUCTURE and TESS assignment analyses. The first clade represented *A. saligna* subspecies *'saligna'*, while the second well-supported clade represented *A. saligna* subspecies *'stolonifera'*. The remaining accessions of *A. saligna* subspecies *'lindleyi'* were identified as sister taxa to the *A. saligna* subspecies *'saligna'* clade. The clustering of pairwise genetic distances for nDNA accessions (Appendix S3—Fig. S8B, Supporting information) supported the three clades identified by microsatellite clustering in STRUCTURE.

Statistical parsimony of cpDNA also identified two very divergent lineages within *A. saligna*. However, these divergent lineages were incongruent with the ETS phylogeny in the placement of taxa (Fig. 2). The 722-bp

trnQ-5'rps16 alignment contained only four parsimony-informative sites, and eight distinct haplotypes were identified (Fig. 2B). The first lineage included native reference individuals of *A. saligna* subspecies *'lindleyi'* and *A. saligna* subspecies *'saligna'* as well as introduced individuals from South Africa, South Australia, New South Wales, Israel and Spain (Fig. 2B). The second lineage included native reference individuals of *A. saligna* subspecies *'stolonifera'*, additional individuals from the Busselton population in Western Australia and two individuals from South Australia (Fig. 2B).

Nonmetric multidimensional scaling of pairwise genetic distances for native and introduced populations further illustrated incongruences between the cpDNA and nDNA (Appendix S3—Fig. S8, Supporting information). However, the same incongruences were identified by phylogenetic reconstructions and Bayesian clustering. The two major groups retrieved for cpDNA data clearly differentiated *A. saligna* subspecies *'stolonifera'* from all other native and introduced accessions (Appendix S3—Fig. S8A, Supporting information). Three major native groups were retrieved from the nDNA data representing the subspecies of *A. saligna*, and one additional group (all South African accessions, and accessions from Busselton, Dinninup and Wannerroo in Western Australia, Appendix S3—Fig. S8B, Supporting information).

The PCoA identified similar results to the NMDS analysis (Appendix S3—Figs S5 and S8 respectively, Supporting information). Specifically, the PCoA identified three groups: group one included native populations of *A. saligna* subspecies *'lindleyi'*; group two included populations of *A. saligna* subspecies *'saligna'* and *A. saligna* subspecies *'stolonifera'*; while group three included all South African populations, and Tuart Forest and Busselton populations from Western Australia.

Assessment of genetic variance between the native and introduced ranges showed that a moderate level of microsatellite diversity was partitioned among the native and introduced range (14.5%), while the majority of microsatellite diversity was partitioned within populations (Table 3). A similar pattern was found in the nuclear sequence data, with moderate but lower diversity partitioned among populations (7.7%), and the majority of diversity was partitioned within populations (61.2%, Table 3). Overall, population pairwise F_{ST} values indicate moderate to high differentiation in native populations, and low to moderate population genetic differentiation in the introduced range (Appendix S3—Table S1, Supporting information).

The Bayesian clustering algorithms all broadly separated native and introduced populations, although they identified different numbers of optimal K-clusters (Fig. 1, Appendix S3—Figs S2 to S4, Supporting information). STRUCTURE identified two genetic clusters, broadly corresponding to native and introduced populations (Appendix S3—Fig. S9, Supporting information). Overall, the STRUCTURE analysis showed that all introduced South African populations displayed the closest genetic affinity to populations at Busselton and Tuart Forest from the native range (Fig. 3A, Appendix S3—Figs S5, S7 and S9 Supporting information). GENELAND identified seven clusters and assigned individuals to a genetic cluster with membership coefficients (q_i) of >0.95. GENELAND was also the only algorithm that identified more than one genetic cluster in the introduced populations (Fig. 3). TESS identified five clusters (Appendix S3—Fig. S3B, Supporting information). Similarly to the STRUCTURE results, the TESS analysis showed that introduced populations displayed the strongest genetic affinity to populations from Busselton (assigned with a q_i of >0.7).

Overall, the divergence present within the native subspecies of *A. saligna*, and native and introduced clades

Table 3 A hierarchical AMOVA partitioning of genetic variation in *Acacia saligna* for nuclear microsatellite and nuclear DNA sequence data (nDNA) at various spatial scales: among native and invasive regions; among populations and within populations within native and invasive regions

Source of variation	d.f.	Sum of squares	Variance	Percent variation (%)	Fixation index*
nDNA					
Among native and invasive range	1	800.15	33.546	31.1	0.11118
Among populations	15	1308.29	8.248	7.7	0.38795
Within populations	31	2044.06	65.937	61.2	0.31139
Microsatellite					
Among native and invasive range	1	159.88	0.37722	14.5	0.34044
Among populations	19	362.26	0.51057	19.6	0.22890
Within populations	709	1219.48	1.72000	66.0	0.14465

*All values significant, significance was tested using 10 000 random permutations.

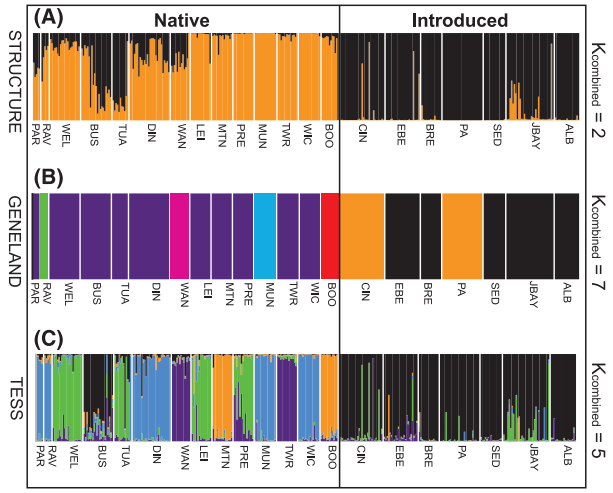

Fig. 3 Identification of the number of distinct genetic groups of *Acacia saligna* in the native (Western Australia) and introduced (South Africa) range using three Bayesian clustering algorithms. The data sets contain a total of 365 individuals genotyped at 10 nuclear microsatellite loci. Membership of each individual's genome (q_i) to the inferred number of genetic clusters is indicated by vertical bars.

was substantially greater than divergences observed within other subspecies or species of *Acacia* (Appendix S3—Table S2, Supporting information). The genetic distance between the native and introduced clade of *A. saligna* (see Fig. 2) was within the same order of magnitude as the genetic distance between the native *A. saligna* subspecies *'lindleyi'* and its closest relative *A. rostellifera* (Appendix S3—Table S2, Supporting information). In addition, the genetic distance between the native subspecies of *A. saligna* was an order of magnitude greater than the genetic distance between the different subspecies of *A. longifolia*.

Discussion

Our results indicate that the introduction efforts of *A. saligna* into South Africa have led to an invasion that is characterized by unstructured, high genetic diversity that is divergent from that found in pure native lineages in Western Australia. Genetic divergence and novelty of this magnitude can arise through numerous processes, including strong drift (e.g. Roy & Buronfosse 2011), postintroduction selection (e.g. Lavergne & Molofsky 2007), admixture (e.g. Kolbe *et al.* 2007) and interspecific hybridization (e.g. Prentis *et al.* 2009).

Both sets of nuclear data (microsatellite and DNA sequence) suggest that admixture between different subspecies has not occurred in South Africa. Indeed, South African populations shared no close relationship with any of the known informal subspecies of *A. saligna* (i.e. reference populations, ETS data). Furthermore, we rule out the possibility that paralogous gene regions may explain the observed patterns as we sequenced multiple cloned gene copies for the ETS region for a number of taxa and never retrieved multiple copies from the same individual from both major clades. It is also unlikely that the invasive lineage represents an unsampled native lineage because: a) we extensively sampled the *A. saligna* complex throughout its distribution in Western Australia; and b) we included populations representative of the three known genetic lineages in our analyses (Millar *et al.* 2011). The South African populations showed relatedness to additionally sampled populations from the native range, one of which (the Busselton population) appears to be cultivated or planted.

Acacia saligna has been widely planted for agroforestry and as a roadside species throughout Western Australia (Maslin & McDonald 2004). Unfortunately, identification of planted stands in the field is very difficult, even for experts (W. O'Sullivan, personal communication). Field inspection of the Western Australian population (Busselton, south of Perth) most closely related to South Africa populations confirmed that this site was indeed planted (B. Maslin and W. O'Sullivan, personal communication). Microsatellite divergence between the same population (Busselton) and pure native lineages of *A. saligna* was too large to assign an existing subspecies identity to this population. Field inspection of other Western Australian populations closely related to South African populations based on nuclear sequence data (i.e. Wanneroo) suggested that these populations may be natural.

Unfortunately, there are no detailed historical records of the location of *A. saligna* plantings in Western Australia, and no information on the source of seed used in these plantings, nor is the source of seeds exported from Australia known. Our genetic results suggest that the origin of South African propagules is the same as the source of Western Australian plantings. The earliest herbarium record of a cultivated *A. saligna* tree is from Western Australia in 1838 in the Swan River region (Royal Botanic Gardens Melbourne, MELISR database, accessed on 18 August 2011). Interestingly, the earliest records of seeds imported to South Africa were at a similar time (in 1833, Poynton 2009). The number of introductions and scale of seeds introduced to South Africa (several thousand to several million; Roux 1961; Poynton 2009) suggest that collections of seeds must have come from large, mature stands likely only present in the native range. In addition, the presence of a central seed distributor in South Africa (Cape Seed

Store, see Poynton 2009) may explain the lack of genetic structure throughout South Africa.

While cultivation could give rise to the genetic differences observed between planted, invasive and native *A. saligna* populations, the incongruence between cpDNA and nDNA phylogenies is currently unexplained. There were no genetic similarities between introduced South African populations and native reference populations of *A. saligna* subspecies *'stolonifera'* at the nDNA or cpDNA gene regions examined (Fig. 2, Appendix S3—Figs S3 and S8, Supporting information). However, South African individuals and native individuals of *A. saligna* subspecies *'lindleyi'*, *'pruinescens'* and *'saligna'* (Millar *et al.* 2011) appeared to be related (cpDNA, Fig. 2A). It is thus unlikely that South African populations originated from populations of *A. saligna* subspecies *'stolonifera'* in Western Australia, but may have originated from hybridization between a number of parental lineages of *A. saligna* subspecies *'lindleyi'*, *'pruinescens'* and *'saligna'*. Although discrepancies between nuclear and chloroplast phylogenies can be caused by a variety of factors [e.g. lineage sorting of ancestral polymorphisms or nonhomologous sampling of duplicated genes (unlikely as we included multiple clones accessions of the nuclear gene)], we suggest that our results most likely represent introgressive hybridization (hybridization followed by backcrossing) and led to chloroplast capture within *A. saligna*.

If introgression had occurred between parental lineages of *A. saligna* (subspecies *'lindleyi'*, *'pruinescens'* and *'saligna'*) and a closely related, but currently unknown species, then we would expect that the genetic distance between the South African clade and different subspecies of *A. saligna* would be substantial. This is precisely what we observed in the nuclear DNA. The genetic distance between the South African clade and the native clade was approximately the same order of magnitude to the distance between native *A. saligna* and its sister taxa, *A. rostellifera* (Table S2). Indeed, the distance between the native and introduced clade far exceeds our observations (based on available data retrieved from GenBank) of divergences between subspecies of Australian acacias (but see Wardill *et al.* 2005) and is on a level with divergences at the species level. Our microsatellite analyses also support this hypothesis. It appears that the divergence between native and introduced ranges is largely driven by private alleles present within each range despite a thorough sampling of native populations. This is further supported when considering differences in allele frequencies, with one-fifth of all alleles that differed by more than 10% in their frequencies between ranges, being private alleles restricted to the native range.

Many species that are now invasive were introduced to new regions for their economic value. Such species have been subject to cultivation and breeding practices to artificially select advantageous traits to promote faster growth rates or higher biomass production. Consequently, cultivated genotypes present in the introduced range may be fitter than their native counterparts (e.g. Lavergne & Molofsky 2007), and these may pose a greater threat as an invasive species (e.g. *Mahonia aquifolium*; Ross & Auge 2008). A number of successful invaders have been subject to some form of cultivation or breeding that may have facilitated persistence in a new environment (Ross 2009). Thus, the selection pressures imposed on a species by cultivation may play a substantial role in invasive success.

We recommend that future research should focus on comparing quantitative and qualitative traits of native and invasive genotypes of *A. saligna* under common garden conditions. Such experiments would allow the testing of native genetic variation in concert with heritable phenotypic variation. Furthermore, the genetic dissimilarity of native and introduced populations may be related to possible increased fitness effects of cultivation in the native range.

In agreement with our genetic data, previous work has shown that the subspecies of *A. saligna* differ dramatically in the bioclimatic niches they occupy in Western Australia and their potential range in South Africa (Thompson *et al.* 2011). The novel genetic entity identified here means that predictions of potential range size using environmental tolerances of genetic entities in the native range will be inaccurate. In such cases, where the taxon has had sufficient residence time to sample potential invasible sites, predictions based on introduced environmental distribution correlates are likely to offer better results (Rouget *et al.* 2004). Clearly, the assumption that introduced taxa lumped under the name of *'A. saligna'* will perform similarly throughout their introduced range is problematic.

The dissimilarity in genetic composition between the native and introduced range and habit of biological control agents could significantly affect the overall success of control programmes. Assuming that genetic similarity will translate into host specificity, our findings suggest that the biocontrol agent (*U. tepperianum*) was, perhaps fortuitously, collected from a suitable Western Australian source (Busselton) of *A. saligna*. The suggested common garden experiments, including pathogenicity and host specificity tests on various sources of *U. tepperianum* from Western Australia, may further enhance control in South Africa.

In summary, our results show how cultivation, the number and size of introduction events, human-mediated transport, genetic drift and possibly introgres-

sive hybridization can act swiftly and concurrently to create genotypic novelty. Such genotypic novelty has important implications for management, for example, in predicting potential range and assessing options for classical biological control. In the absence of such a holistic approach, we have demonstrated that taxonomic identity and biogeographic provenance(s) alone, aspects crucial for the initial implementation of successful management, can easily lead to erroneous deductions. Our study not only shows the value of using different molecular approaches to understand invasion histories, but also raises the fundamental question of whether (and how quickly) introduced species can be regarded as fundamentally different entities to their native counterparts (Müller-Schärer *et al.* 2004).

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J.R.U.W, D.M.R, M.B and M.A.M initiated the collaboration. G.D.T, J.J. Le Roux, J.R.U.W and D.M.R conceived the research ideas. G.D.T and J.J. Le Roux designed the research methods. M.B and M.A.M contributed genomic DNA. G.D.T. generated and analysed all molecular data and led the writing.

Data accessibility

GenBank numbers: JF273799–JF273827, JQ812744–JQ812795. All sequence and microsatellite data contained within DRYAD entry: doi: 10.5061/dryad.hr3dc177.

Supporting information

Additional Supporting Information may be found in the online version of this article.

Appendix S1 Methods for amplification of gene regions.

Appendix S2 Bayesian clustering methods.

Appendix S3 Additional tables and figures from results.

Fig. S1 Isolation by distance analysis for native and introduced populations of *A. saligna*.

Fig. S2 Principal Component analysis of microsatellite data.

Fig. S3 Bayesian clustering of native populations using GENELAND and TESS.

Fig. S4 Identification of the number of clusters in the native and introduced range using TESS.

Fig. S5 Hierarchical clustering in the native range using STRUCTURE.

Fig. S6 Native and introduced allelic frequencies.

Fig. S7 nDNA statistical parsimony network of native and introduced *A. saligna* accessions.

Fig. S8 Multi-dimensional scaling of ETS and trnQ data.

Fig. S9 Hierarchical clustering in the native and introduced range using STRUCTURE.

Table S1 Population genetic structure in the native and introduced range.

Table S2 Genetic distances between species and subspecies of acacias.

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